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Immunohistochemical study of α1-5 chains of type IV collagen in hereditary nephritis

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Immunohistochemical study of α1-5 chains of type IV collagen in hereditary nephritis. The distribution of α1-5 chains of type IV collagen [α1-5(IV)] in the glomerular basement membrane (GBM) and epidermal basement membrane (EBM) of 23 families with hereditary nephritis was examined by indirect immunofluorescence. These families were divided into three clinicopathological groups. Group I (10 families) patients showed a widespread "basket weave" pattern of the GBM and a family history of nephritis was present. Group II (6 families) patients showed a widespread "basket weave" change without a family history of nephritis. Group III (7 families) patients showed a widespread attenuation of the GBM but no "basket weave" change, and had a family history of nephritis and chronic renal failure. α1(IV) and α2(IV) were present in all affected and unaffected family members and controls. All normal family members and controls expressed α3(IV), α4(IV) and α5(IV) in the GBM and α5(IV) in the EBM in a diffuse pattern. All group I families and three of the group II families exhibited complete loss of the α5(IV) antigen from the GBM and EBM in male patients, and segmental loss of the α5(IV) antigen in female patients. In these families the α3(IV) and α4(IV) antigens were completely lost from the GBM in male patients with severe nephritis, whereas α3(IV) and α4(IV) were present but diminished in male patients with mild nephritis. Three group II and all group III families expressed the α3-5(IV) antigens in an identical manner to that of normal controls. These findings indicate that the heterogeneity of hereditary nephritis reflects a variety of aberrant expression patterns of α3-5(IV) and that immunohistochemical examination of α5(IV) in the EBM is a useful method for the diagnosis of X-linked Alport syndrome.

Hereditary nephritis is a familial disorder which generally has a progressive course. It is usually present with hematuria in childhood, and when associated with neurosensory deafness it is known as Alport syndrome [1]. Characteristic alterations of the glomerular basement membrane (GBM) are observed on electron microscopy [2–4]. There is irregular thickening of the GBM with replication of the lamina densa, forming a "basket weave" pattern, and enclosing electron-lucent lacunae which frequently contain small dense particles. When diffuse, these changes are diagnostic of Alport syndrome [5]. A widespread basket weave pattern of the GBM has also been reported in patients with hematuria but no family history of nephritis, and it has been suggested that such GBM changes in patients with nonfamilial hematuria may represent new mutations of Alport syndrome [6–8]. However, a widespread basket weave GBM pattern is not present in all patients with hereditary nephritis; some patients show a widespread attenuation of the GBM instead [9].

Alport syndrome is a genetically heterogeneous renal disease. X-linked dominant, autosomal dominant and autosomal recessive modes of inheritance have been reported [10–12]. However, the majority of Alport pedigrees published show X-linked dominant inheritance [11].

The characteristic GBM alterations in patients with Alport syndrome suggest that the defect is expressed in one of the structural components of the GBM. Abnormalities in the type IV collagen of the GBM have been heavily implicated in the pathogenesis of Alport syndrome. The type IV collagen molecule has a triple-helical structure composed of 3 α chains [13]. There are five isoforms of these chains in the GBM: α1(IV), α2(IV), α3(IV), α4(IV) and α5(IV). Recently the gene for a sixth chain, α6(IV), has been identified [14]. These triple-helical type IV collagen molecules comprise the GBM network [15]. Alterations of the Goodpasture antigen and the Alport antigen in the GBM have been reported in Alport patients [10, 16–21], as have alterations of the Alport antigen in the epidermal basement membrane (EBM) [22, 23]. Goodpasture antigen is localized to the NC1 domain of α3(IV) [24, 25], while the Alport antigen and the NC1 domain of α5(IV) may be the same or homologous molecules [26]. The α3(IV) gene maps to chromosome 2 [27] and the α5(IV) gene maps to the long arm of the X chromosome [28]. Mutations in the α5(IV) gene have been reported in X-linked Alport syndrome [29, 30], and recently mutations in the α3(IV) and α4(IV) genes have been identified in autosomal recessive Alport syndrome [31].

Since very little data about the proteins are available for α chains of type IV collagen in hereditary nephritis, in the present study we examined the distribution of α1(IV), α2(IV), α3(IV), α4(IV) and α5(IV) in the GBM and EBM of patients with hereditary nephritis using indirect immunofluorescence, and here we discuss the relationship of the distribution of these chains to the clinical and pathological features.

Methods

Families

Forty-four members from 23 families with familial nephritis were examined after obtaining their informed consent. These families were divided into three clinicopathological groups.

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Group I (families OK, OG, KA, IN, TO, HO, KO, YA, SA, and MA). Patients showed a widespread "basket weave" pattern of the GBM, and a family history of nephritis was present.

Group II (families SI, OA, YO, SM, TE and SB). Patients showed a widespread "basket weave" pattern of the GBM, but a family history of nephritis was absent.

Group III (families UM, NI, MR, YG, NO, MK and OO). Patients showed a widespread attenuation of the GBM but no "basket weave" pattern. Attenuation of the GBM was defined as a thickness of less than 250 nm [32]. A family history of nephritis and chronic renal failure was present.

The clinical and electron microscopic features are summarized in Table 1.

In group I, each family exhibited an inheritance pattern compatible with X-linked transmission; there was mother-to-son or mother-to-daughter transmission, absence of father-to-son transmission, and nephritis was more severe in male patients than in females. However, it was often difficult to exclude the possibility of an autosomal inheritance mode from the family history as the families were small. In four families, neurosensory deafness was present in male patients with nephritis. Cataract was found in male patients in two families, but no other ocular anomalies were found in group I patients with nephritis. In six families, progression to renal failure was observed in male patients and a male patient in family OG received a kidney transplant from his father. He has not developed post-transplant anti-GBM nephritis. The age at onset of end-stage renal disease (ESRD) was less than 20 years in five families, but more than 30 years in one family. In families KA, TO and YA there was only one male patient in each family and nephritis appeared severe in these male patients. In families SA and MA nephritis appeared milder in male patients. First renal biopsy of a male patient in family MA at the age of seven years showed widespread attenuation of the GBM without a basket weave change, but his second biopsy at the age of 15 years showed a widespread "basket weave" pattern.

In group II only a single subject was affected in each family. No family exhibited consanguinity. There were two male and four female patients with nephritis. Four patients showed neurosensory deafness. One male patient from family SI had a cataract, but no other ocular anomalies were found in group II patients. Two patients developed ESRD before the age of 20 years and received kidney transplants from their parents. They have not developed post-transplant anti-GBM nephritis.

In group III, neurosensory deafness was present in three families. No ocular anomalies were found in group III patients with nephritis. The age at onset of ESRD was more than 30 years in all families. Figure 1 shows pedigrees of group III families.

Tissue

Kidney tissues were obtained by percutaneous renal biopsy, using a Tru-Cut needle under X-ray control or ultrasonic guide, from 25 patients belonging to 22 families. Renal tissues from normal subjects in families OG, SI and SM were obtained from transplanted kidneys at the time of renal transplantation. Five other normal renal biopsy specimens obtained at the time of renal transplantation were used as normal controls.

Skin specimens (4 mm in diameter) were obtained by punch biopsy from the volar aspect of the forearm from 37 members of 18 families. Skin biopsy specimens obtained from one healthy volunteer and two patients with other renal diseases, and histologically normal specimens of skin obtained from four individuals were used as normal controls. Both kidney and skin were studied in 17 families, kidney alone in five, and skin alone in one.

Antibodies

Polyclonal antibodies anti-α1(IV), anti-α2(IV), and anti-α3(IV), and mouse monoclonal antibody to anti-α3(IV) NC1 (MAb17) [33–35] were provided by Dr. J. Wieslander (Statens Seruminstitut, Copenhagen, Denmark). The polyclonal antibodies were obtained from rabbits immunized with bovine NC1 domain [33, 36]. Monoclonal anti-α4(IV) NC1 antibody (MAb85) obtained from mice was provided by Dr. R.J. Butkowski (University of Minnesota, Minnesota, USA) [36]. Another monoclonal anti-α4(IV) NC1 peptide antibody (MK4) was also used. This was made by immunizing mice with a rat NC1 fraction [37] and reacted with a derived non-consensus amino acid sequence (PAPDTLKESQ) [38]. Monoclonal anti-α5(IV) NC1 peptide antibody (H51) was also made by immunizing rats with a derived non-consensus amino acid sequence (VDVSDMFSKPQSE) [39]. MAb85 was diluted to 1:20 with PBS for the use of immunohistochemical study. The other antibodies were not diluted for the use of immunohistochemical study.

Indirect immunofluorescence

Kidney and skin tissues were snap-frozen in a dry ice and acetone bath. They were cut into 4 μm sections, air dried, fixed in 95% ethanol at 4°C for five minutes, and rinsed three times with PBS at room temperature. Kidney and skin tissue sections for immunostaining with primary antibodies against α3(IV), α4(IV) and α5(IV) were denatured in 6 M urea, 0.1 M glycine HC1 buffer, pH 3.5, at 4°C for one hour [40], and then washed three times with PBS prior to reacting with primary antibodies. The sections were stained by an indirect method using the primary antibodies against α1(IV), α2(IV), α3(IV), α4(IV) and α5(IV). After reaction for 45 minutes at room temperature, secondary antibodies, fluorescein isothiocyanate (FITC)-conjugated goat [F(ab')2] anti-rat IgG, FITC-conjugated goat [F(ab')2] anti-mouse IgG and FITC-conjugated goat [F(ab')2] anti-rabbit IgG (Organon Teknika-Cappel, Durham, North Carolina, USA) were added. Secondary antibodies were diluted to 1:60 with PBS before the use. The sections were viewed with an Olympus BH2-RFCA reflecting microscope (Olympus Optical Co., Tokyo, Japan).

To demonstrate the relationship between glomerular α3(IV) or α4(IV) and α5(IV) distribution in a group I female patient, dual fluorochrome immunofluorescence was carried out. Renal tissue was first stained with anti-α5(IV) antibody, which was detected with FITC-conjugated goat [F(ab')2] anti-rat IgG, and then stained with anti-α3(IV) or anti-α4(IV) antibody which was detected with rhodamine-conjugated goat [F(ab')2] anti-rabbit IgG or rhodamine-conjugated goat [F(ab')2] anti-mouse IgG, respectively (Organon Teknika-Cappel).

Results

In glomeruli from normal control kidney tissues α1(IV) and α2(IV) were distributed in a diffuse pattern through the GBM and mesangium, while α3(IV), α4(IV) and α5(IV) were confined to the GBM. In normal control skin tissues α1(IV), α2(IV) and α5(IV) were diffusely distributed in the EBM, but α3(IV) and α4(IV) were absent in the EBM (Fig. 6).

Table 1. Clinicopathological findings and distribution of α3, 4 and 5 chains of type IV collagen in the glomerular basement membrane and epidermal basement membrane

Family	Family history of nephritis	Deafness	Cataract	End-stage renal disease (onset age)	GBM changes	Affected males: Number of subjects examined	α3	α4	α5		Affected females: Number of subjects examined	α3	α4	α5	
Group I															
OK	+	+	+	+ (<20 years)	basket weave	2	−	−	−	GBM,EBM	1			+s	EBM
OG	+	+	+	+ (<20 years)	basket weave	2	−	−	−	GBM,EBM					
KA	+	+			basket weave	1	−	−	−	GBM,EBM	1			+s	EBM
IN	+	+		+ (<20 years)	basket weave						2	+s	+s	+s	GBM,EBM
TO	+				basket weave	1	−	−	−	GBM					
HO	+			+ (<20 years)	basket weave						3	+s	+s	+s	GBM,EBM
KO	+			+ (<20 years)	basket weave						3			+s	EBM
YA	+				basket weave	1	−	−	−	GBM,EBM	1			+s	EBM
SA	+				basket weave	1	±d	±d	−	GBM,EBM	1			+s	EBM
MA	+			+ (>30 years)	basket weave	1	±d	±d	−	GBM,EBM	1			+s	EBM
Group II															
SI		+	+	+ (<20 years)	basket weave	1	−	−	−	GBM,EBM					
OA		+			basket weave	1	−	−	−	GBM,EBM					
YO					basket weave						1	+s	+s	+s	GBM,EBM
SM		+		+ (<20 years)	basket weave						1			+d	EBM
TE		+			basket weave						1	+d	+d	+d	GBM,EBM
SB					basket weave						1	+d	+d	+d	GBM
Group III															
UM	+	+		+ (>30 years)	attenuation	1	+d	+d	+d	GBM	1	+d	+d	+d	GBM
NI	+	+		+ (>30 years)	attenuation	1	+d	+d	+d	GBM					
MR	+	+		+ (>30 years)	attenuation	1	+d	+d	+d	GBM,EBM					
YG	+			+ (>30 years)	attenuation	1	+d	+d	+d	GBM,EBM	1			+d	EBM
NO	+			+ (>30 years)	attenuation						1	+d	+d	+d	GBM,EBM
MK	+			+ (>30 years)	attenuation						1	+d	+d	+d	GBM
OO	+			+ (>30 years)	attenuation	1	+d	+d	+d	GBM					

Family	Normal males: Number of subjects examined	α3	α4	α5		Normal females: Number of subjects examined	α3	α4	α5	
Group I										
OK										
OG	1	+d	+d	+d	GBM,EBM					
KA										
IN										
TO										
HO										
KO	1			+d	EBM					
YA										
SA										
MA										
Group II										
SI	1	+d	+d	+d	GBM	1			+d	EBM
OA						1			+d	EBM
YO						1			+d	EBM
SM						1	+d	+d	+d	GBM,EBM
TE										
SB										

Abbreviations are: +, strongly positive staining; ±, slightly positive staining; −, negative staining; d, diffuse distribution; s, segmental distribution; GBM, glomerular basement membrane; EBM, epidermal basement membrane.

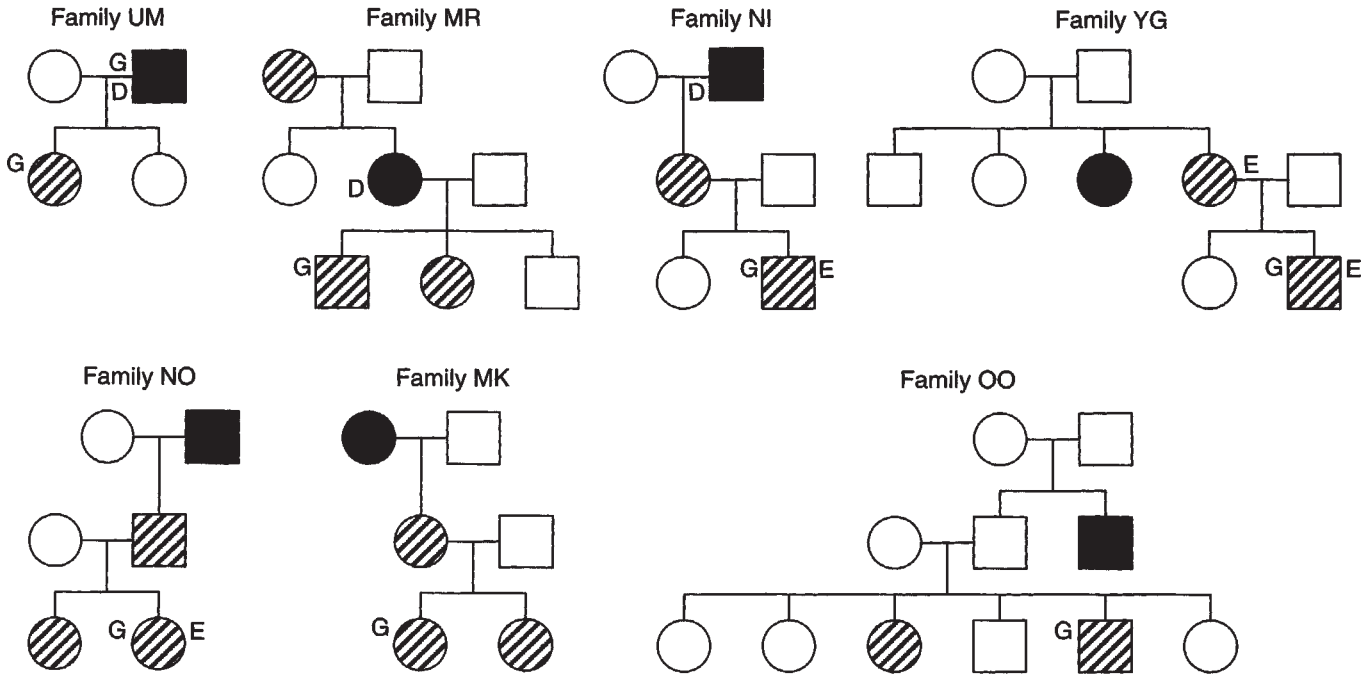

Fig. 1. *Pedigrees in group III families.* Squares (□) and circles (○) denote males and females, respectively. Solid symbols (■ ●) denote individuals with end-stage renal disease and white cross-hatched symbols (▨ ▨) indicate individuals with hematuria. G indicates subjects who had renal biopsy and E indicates subjects who had skin biopsy. D indicates subject with deafness.

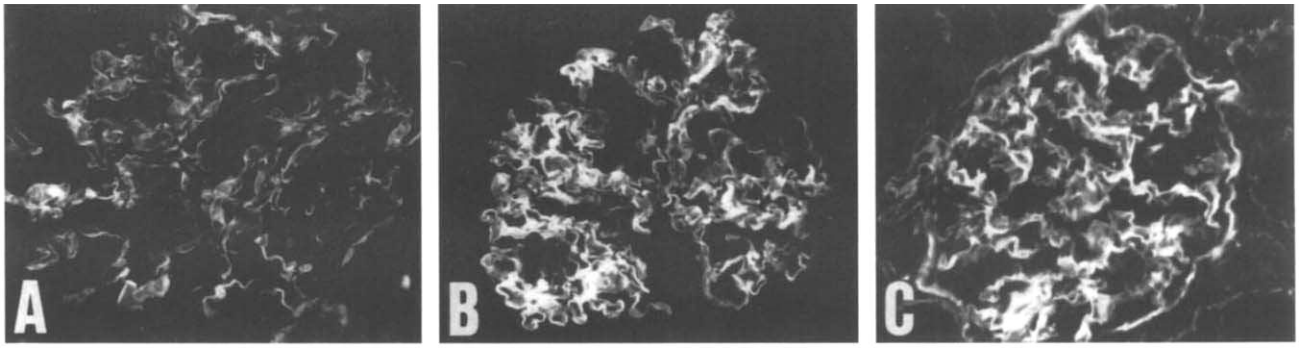

Fig. 2. *Normal glomeruli stained with anti-α3(IV)* (**A**)*, anti-α4(IV)* (**B**) *and anti-α5(IV)* (**C**) *antibodies.* There is diffuse linear binding of anti-α3(IV), anti-α4(IV) and anti-α5(IV) antibodies to the GBM (×270).

α1(IV) and α2(IV) were clearly detected along the GBM and EBM in all family members examined.

The results of the indirect immunofluorescence studies of α3(IV), α4(IV) and α5(IV) in the GBM and EBM are presented in Table 1. A diffuse pattern of α3(IV), α4(IV) and α5(IV) was observed in the GBM of all normal family members examined (Fig. 2), and diffusely distributed α5(IV) was also present in the EBM of all normal family members examined (Fig. 6C).

In group I all male patients showed complete absence of the α5(IV) antigen from the GBM (Figs. 3C and 4C) and EBM (Fig. 7A). A segmental distribution of the α5(IV) antigen was observed in the GBM (Fig. 5C) and EBM (Fig. 7B) of all female patients. The male patients from families OK, OG, KA, TO and YA showed complete absence of the α3(IV) and α4(IV) antigens from the GBM (Fig. 3A and B), whereas diminished but diffuse staining of the GBM with anti-α3(IV) and α4(IV) antibodies was demonstrated in male patients from families SA and MA, in which nephritis appeared milder (Fig. 4A and B). Female patients from families IN and HO exhibited a segmental distribution of α3(IV) and α4(IV) in the GBM (Fig. 5A and B). Dual label immunofluorescence in a female patient from family HO demonstrated that α3(IV) and α5(IV) were similarly distributed in her GBM, as were α4(IV) and α5(IV) (Fig. 8). Thus, α5(IV) was codistributed with α3(IV) and α4(IV) in this patient's GBM.

In group II, α5(IV) expression in both the GBM and EBM was completely absent in male patients from families SI and OA, and a segmental α5(IV) distribution was observed in the GBM and EBM of the female patient from family YO. The male patients from families SI and OA also showed a complete absence of the α3(IV) and α4(IV) antigens from the GBM, while the female patient from family YO showed a segmental distribution of α3(IV) and α4(IV). The expression pattern of the α3(IV), α4(IV)

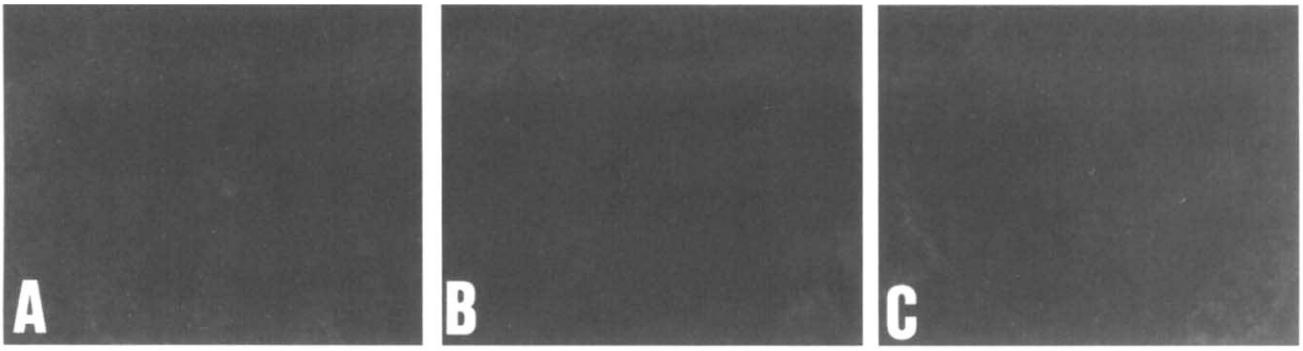

Fig. 3. *Glomeruli of a group I male patient with severe nephritis (family OK) stained with anti-α3(IV)* (**A**)*, anti-α4(IV)* (**B**) *and anti-α5(IV)* (**C**) *antibodies.* No binding of anti-α3(IV), anti-α4(IV) or anti-α5(IV) antibodies to the GBM was observed (×270).

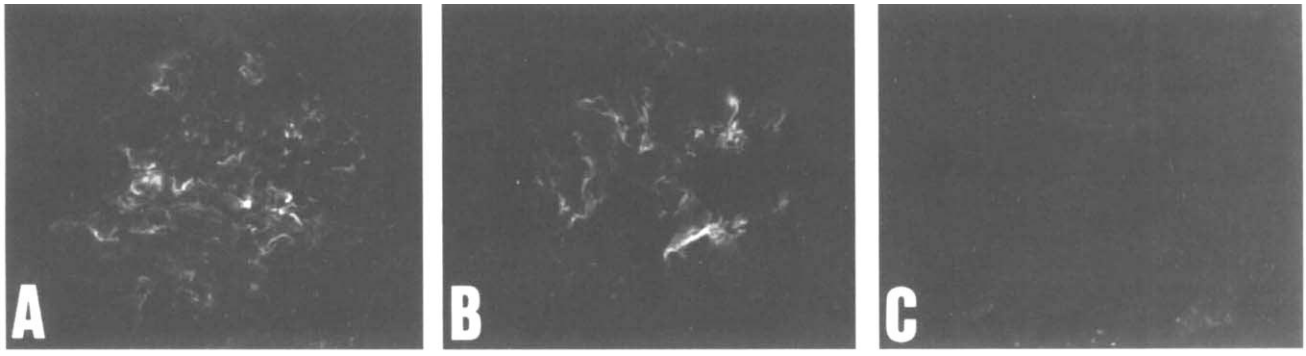

Fig. 4. *Glomeruli of a group I male patient with mild nephritis (family SA) stained with anti-α3(IV)* (**A**)*, anti-α4(IV)* (**B**) *and anti-α5(IV)* (**C**) *antibodies.* There was diminished binding of anti-α3(IV) and anti-α4(IV) to the GBM, but no binding of anti-α5(IV) antibody (×270).

Fig. 5. *Glomeruli of a group I female patient (family HO) stained with anti-α3(IV)* (**A**)*, anti-α4(IV)* (**B**) *and anti-α5(IV)* (**C**) *antibodies.* There was segmental linear binding of anti-α3(IV), anti-α4(IV) and anti-α5(IV) antibodies to the GBM (×270).

and α5(IV) antigens in these three patients from group II was therefore identical to that in the group I patients. The mothers of the three group II patients with abnormal α5(IV) expression showed normal expression of α5(IV) in the EBM. The female patients from the other three families in group II demonstrated normal α5(IV) expression in both the GBM and EBM. These female patients also showed normal expression of α3(IV) and α4(IV) as well as α5(IV) in the GBM.

Families in group III expressed α5(IV) in the GBM and EBM, and α3(IV) and α4(IV) in the GBM, in an identical pattern to that of normal controls.

α1(IV) and α2(IV) were also localized in Bowman's capsule, the tubular basement membrane and the vascular basement membrane of kidney tissues from normal controls and all affected and unaffected individuals examined. α3(IV) and α4(IV) were also distributed occasionally in the tubular basement membrane and segments of Bowman's capsule of normal control kidney tissues. α5(IV) was also detected in Bowman's capsule and the basement membranes of some tubules of normal control kidney tissues. The expression patterns of α3(IV), α4(IV) and α5(IV) in Bowman's capsule and the tubular basement membrane, and the GBM were always identical in affected and unaffected individuals examined.

Skin tissues of four male and two female patients from families OK, OG, IN, HO, MA and SI, and two normal controls were examined by electron microscopy. These six patients showed

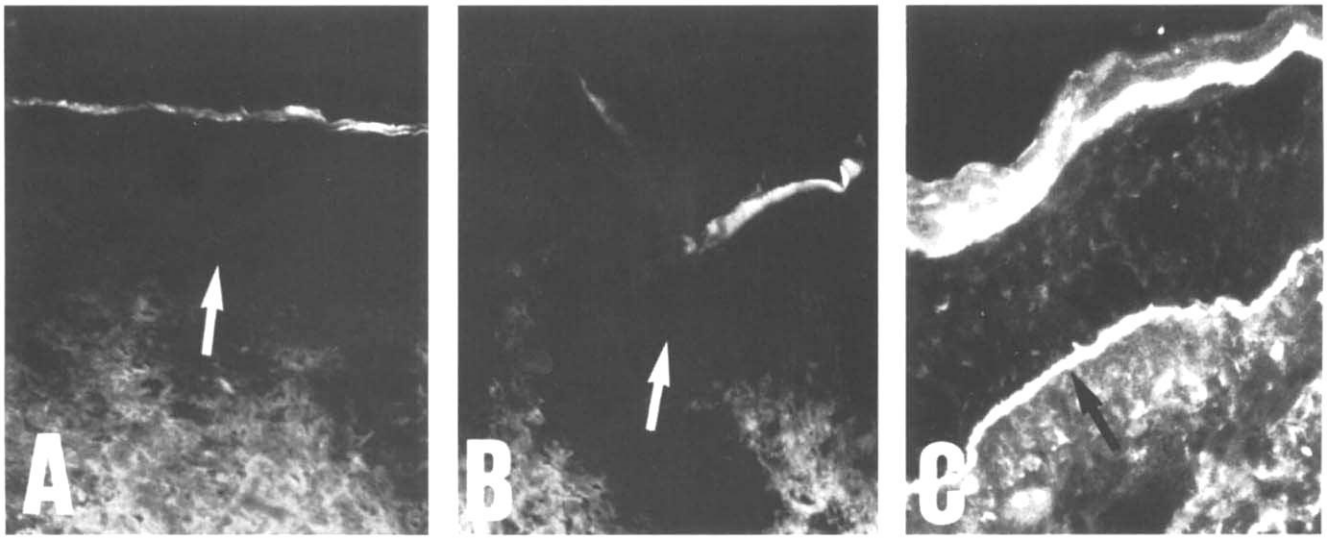

Fig. 6. *Normal skin biopsy specimens stained with anti-α3(IV)* (**A**), *anti-α4(IV)* (**B**) *and anti-α5(IV)* (**C**) *antibodies.* There is diffuse linear binding of anti-α5(IV) antibody to the epidermal basement membrane (EBM) (black arrow), but no binding of anti-α3(IV) and anti-α4(IV) antibodies to the EBM is observed (white arrow) (×410).

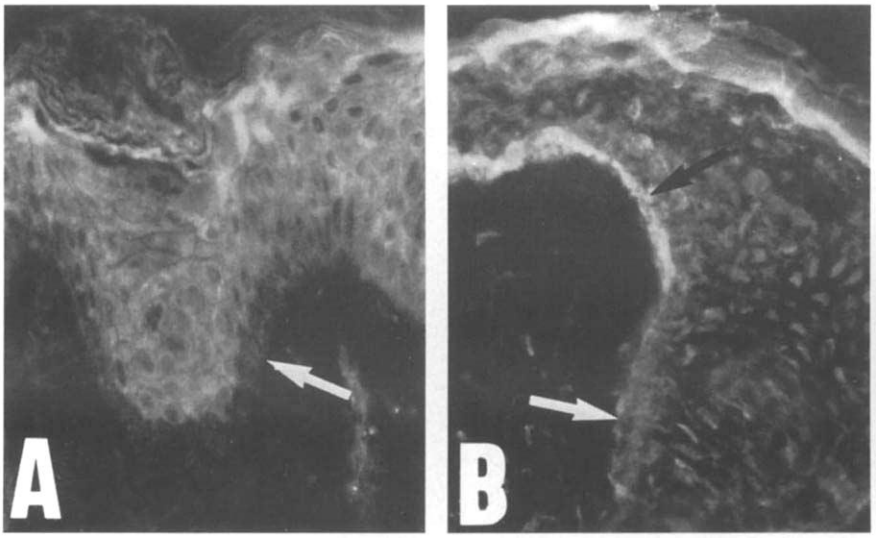

Fig. 7. *Skin biopsy specimens from a group I male patient (family OK)* (**A**) *and a group I female patient (family OK)* (**B**) *stained with anti-α5(IV) antibody.* No binding of anti-α5(IV) antibody to the EBM (white arrow) was observed in the male patient's skin (**A**) (×410). There was segmental binding of anti-α5(IV) antibody to the EBM in the female patient's skin (**B**) (×410). Note normal binding areas (black arrow) and no binding areas (white arrow) of the EBM.

abnormal expression of α5(IV) in the GBM and EBM and a widespread "basket weave" pattern of the GBM. The EBM was not different among these specimens (Fig. 9). The "basket weave" changes in the EBM were not observed.

Discussion

Alterations in antigenicity of the GBM have been previously reported in Alport syndrome [10, 16–23, 39, 41, 42]. After renal transplantation, some patients with Alport syndrome have developed anti-GBM nephritis in their renal allografts [17, 19, 21]. The patients' anti-GBM sera reacted with control kidneys, but not with the native kidney or the kidneys of other patients with Alport syndrome [17]. IgG in sera from patients with Goodpasture syndrome binds to normal GBM, but not to the GBM of some patients with Alport syndrome [16–18, 20]. These findings suggest the absence of GBM antigens, Alport and Goodpasture antigens in some patients with Alport syndrome. The Goodpasture antigen is located in the NC1 domain of α3(IV) [24, 25], while the Alport antigen and the NC1 domain of α5(IV) are possibly the same or homologous molecules [26].

Families in group I are typical examples of Alport syndrome since a widespread basket weave pattern is present in the GBM of the patients, and nephritis is present in at least two members of each family. Four families (families OK, OG, KA and IN), in which neurosensory deafness is also present, are classical examples of Alport syndrome. All group I families showed an abnormal expression of α5(IV). Unaffected family members showed diffuse linear expression of α5(IV) in the GBM and EBM, however, male patients showed no reactivity of the GBM and EBM with anti-α5(IV) antibody and female patients exhibited segmental reactivity. The distribution of this α5(IV) defect is consistent with X-linked dominant transmission of an abnormal gene, and the

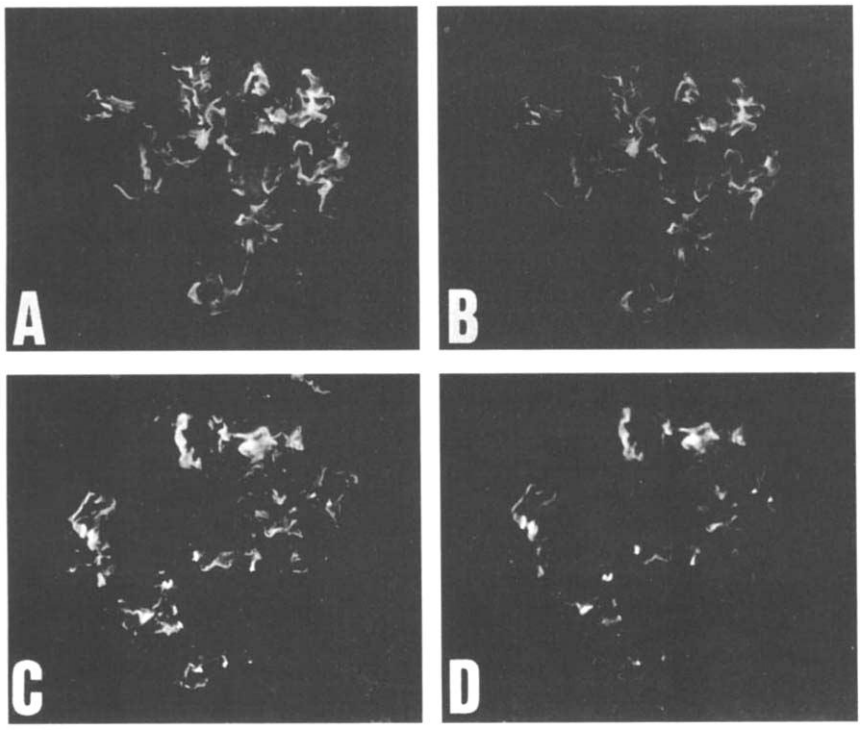

Fig. 8. *Dual label immunofluorescence in a group I female patient (family HO) stained with anti-α3(IV)* (**A**) *and anti-α5(IV)* (**B**) *antibodies, and anti-α4(IV)* (**C**) *and anti-α5(IV)* (**D**) *antibodies.* α3(IV) and α5(IV) were similarly distributed in the GBM, as were α4(IV) and α5(IV) (×270).

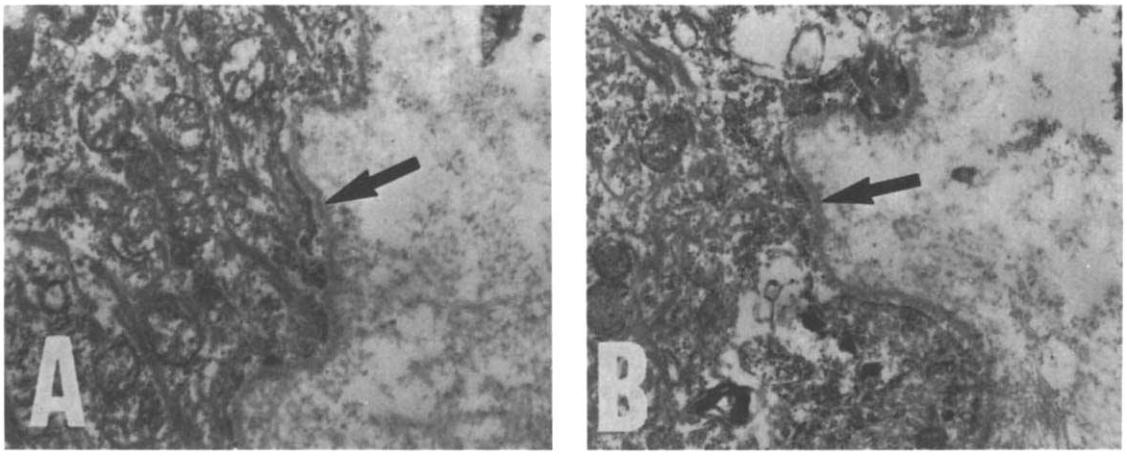

Fig. 9. *Electron micrographs of normal skin* (**A**) *and skin of a group I male patients (family OK)* (**B**). The "basket weave" changes were not observed in the epidermal basement membrane (EBM) (black arrows). There was no apparent abnormalities of the EBM (×16,000).

α5(IV) gene is known to be located on the X-chromosome. Male patients, who only possess the abnormal α5(IV) gene, produce only abnormal α5(IV) which is not detected by anti-α5(IV) monoclonal antibody. The α5(IV)-producing cell in female patients may have either an active normal α5(IV) gene or an active abnormal gene, depending on which X chromosome is randomly inactivated [22]. As a result, female patients have a mixture of normal and abnormal α5(IV). Furthermore, a number of mutations have been found in the α5(IV) gene in families with Alport syndrome [29, 30]. Therefore all the families in group I were considered to have an X-linked type inheritance mode. Autosomal dominant and autosomal recessive modes of inheritance have been reported [10–12], and recently mutations in the α3(IV) and α4(IV) genes have been identified in autosomal recessive Alport syndrome [31]. However, the present findings indicate that a majority of families with Alport syndrome show an abnormal expression of α5(IV) and an X-linked type of inheritance mode.

Patients in group II did not appear to have hereditary nephritis, because nephritis was lacking in their families. However, in our previous study, children with a widespread basket weave pattern of the GBM, with or without a family history of nephritis, showed a tendency to show a progressive disease course, more frequent occurrence of neurosensory deafness, and a more severe prognosis in boys [8]. We were not able to identify any other clinical or pathological features that distinguish such nonfamilial patients from Alport patients. Three patients in group II showed abnormal expression of α5(IV) identical with the abnormal expression observed in group I. Their mothers showed normal α5(IV)

expression. We suspect that these three patients may represent new mutations in X-linked Alport syndrome. Analysis of the α5(IV) gene revealed a mutation in a female patient from family YO. This girl patient showed a segmental α5(IV) distribution in the GBM and EBM.

In the present study, abnormal expression of the α5(IV) antigen was always associated with abnormal expression of the α3(IV) and α4(IV) antigens in the GBM. Male patients showed complete loss of the α3(IV) and α4(IV) antigens in the families with severe disease and diminished expression in the families with mild disease. Female patients showed segmental expression of the α3(IV) and α4(IV) antigens. Dual-label immunofluorescence demonstrated that the α3(IV), α4(IV) and α5(IV) antigens were codistributed in the GBM. Abnormal expression of the α5(IV) antigen in the skin, where α3(IV) and α4(IV) are normally absent, and the presence of α3(IV) and α4(IV) in the glomeruli of some male patients without α5(IV) antigen suggest that a defect of α5(IV) leads to defect of α3(IV) and α4(IV) in the GBM. The mechanism of the abnormal α3(IV) and α4(IV) expression in patients with abnormal α5(IV) has been discussed by Kashtan et al [43] and Reeders [44], but remains to be established. Although primary abnormalities may be present in the α5(IV) gene, the severity of the disease is associated with the expression of α3(IV) and α4(IV). Gubler, Antignac and Knebelmann [45] recently reported that absence of α3(IV) antigen was a marker of severe juvenile forms of Alport syndrome.

In contrast to group I patients, all patients in group III showed normal expression of the α3(IV), α4(IV) and 5(IV) antigens. Group III patients were characterized by a thin GBM. The relationship between thin GBM disease and Alport syndrome is unknown. Progression may be evident from thinning of the GBM to basket weave changes in the GBM in some patients, as in our male patient of family MA in group I. His first biopsy at the age of seven years showed widespread attenuation of the GBM without a basket weave change, but his second biopsy at the age of 15 years showed a widespread basket weave change. Therefore, it is possible that thin GBM disease and Alport syndrome may be variants of the same disease [44]. However, the present study showed that thin GBM disease and Alport syndrome are different diseases in the majority of patients.

The expression patterns of α5(IV) in the GBM and EBM were always identical. Therefore immunohistochemical examination of skin biopsy specimens could be of diagnostic value. A diagnosis of X-linked Alport syndrome can be made by means of skin biopsy using anti-α5(IV) antibody in male patients. However, the use of skin biopsy for X-linked Alport syndrome should be cautious in female patients with hematuria. Because of mosaicism a normal result does not unequivocally exclude heterozygosity. The basket weave changes in the EBM were not observed in patients with a widespread basket weave pattern of the GBM. Our observations agree with the observations of Kashtan et al [46], who also found no apparent abnormalities in the EBM of Alport patients.

In conclusion, the heterogeneity of hereditary nephritis reflects the variety of aberrant expression of α3(IV), α4(IV) and α5(IV), and immunohistochemical examination of α5(IV) in the EBM is useful for diagnosis of X-linked Alport syndrome.

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